THE GRAPERESEQ 18K VITIS GENOTYPING CHIP

M-C Le Paslier[1], N. Choisne[2], S. Scalabrin[3], R Bacilieri[4], A. Berard[1], R Bounon[1,5], J-M Boursiquot[4], M Bras[2], D Brunel[1], A. Chauveau[1], G Di Gaspero[3], L Hausmann[6], T Lacombe[4], V Laucou[4], A Launay[3], JM Martinez-Zapater[7], M Morgante[3], A. Berard[1], H Quesneville[2], R Töpfer[6], R Torres-Perez[7], A-F Adam-Blondon[2,5]

[1] INRA, US1279 EPGV, CEA-IG/CNG, 2 rue Gaston Crémieux, BP 5724, 91057 Evry, France
[2] INRA, UR1164 URGI, route de Saint-Cyr, RD 10, 78026 Versailles, France
[3] IGA, via J.Linussio 51, 33100 Udine, Italy
[4] INRA, Montpellier SupAgro, UMR 1334 AGAP, 2 place Pierre Viala, 34060 Montpellier, France
[5] INRA, UMR1165 URGV, 2 rue Gaston Crémieux, BP 5708, 91057 Evry, France
[6] JKI, Institute for Grapevine Breeding Geilweilerhof, 76833 Siebeldingen, Germany
[7] ICVV, CSIC, UR, Gobierno de La Rioja, Madre de Dios 51, 26006, Logroño, Spain

Summary. With the aim to develop a 20K genotyping chip for the international community, 43 *Vitis vinifera ssp vinifera,* 4 *V. vinifera ssp sylvestris,* 3 *V. cinerea* , 3 *V. berlandieri* , 3 *V. aestivalis* , 3 *V. labrusca* , *1 V. lincecumii, 5 M. rotundifolia* genotypes were paired end re-sequenced using Illumina platforms. An average of 4.3 and of 3.4 millions SNP were detected respectively per *V. vinifera* and per other Vitis species genotypes. SNPs were first filtered upon technical criteria: Illumina score >0.9 and class I type. The project aimed at developing two subsets of SNPs for the chip: a *V. vinifera* specific bubset and a general *Vitis* species subset. For the *V. vinifera* subset, SNPs in regions involved in structural variations and repetitions were filtered out and the remaining SNPs were then selected based on their even physical repartition along the genome together with their MAF (Minimum Allele Frequency). For the *Vitis* species subset, SNPs in repeated regions were filtered out and the remaining SNPs were chosen based on their level of heterozygosity and evenly distributed along the genome. In the end, 14,817 *Vitis vinifera* SNPs and 4,978 Vitis species SNPs were selected along with 205 control SNPs to design a 20K grapevine Infinium genotyping chip (http://urgi.versailles.inra.fr/Species/Vitis/GrapeReSeq_Illumina_20K). Illumina designed an 18,071 SNP chip.

1. Chip development

Leaf harvest and DNA prep
43 diverse *Vitis vinifera*
4 *Vitis sylvestris*
3 *V. cinerea*
3 *V. berlandieri*
3 *V. aestivalis*
3 *V. labrusca*
1 *V. lincecumii*
5 *M. rotundifolia*

INRA, JKI, ICVV, IGA
Carrier et al. (2011) An efficient and rapid protocol for plant nuclear DNA preparation suitable for next generation sequencing methods. Am J Botany 98: e13–e15.

DNA quantification
Library prep
Illumina GAII/ HiSeq sequencing

INRA-EPGV/CNG and IGA platforms
README_EPGV_DataTransfer_Illumina_Sequencing.pdf
(http://urgi.versailles.inra.fr/Species/Vitis/GrapeReSeq_Illumina_20K)

Triming for quality

Alignment on the genome seq
SNP detection

URGI and IGA platforms, ICVV
GrapeReSeq_Illumina_20K_SNP_chip_read_me.pdf
(http://urgi.versailles.inra.fr/Species/Vitis/GrapeReSeq_Illumina_20K)

Submission of 20K loci for an Infinium Illumina Grapevine Genotyping Array
V. vinifera: 15022 SNP
V. aestivalis : 1000 SNPs (ae)
V. berlandierii : 1000 SNPs (be)
V. labrusca : 1000 SNPs (lb)
V. cinerea : 1000 SNPs (cn)
V. lincecumi : 400 SNPs (li)
M. rotundifolia : 578 SNPs (mu)

URGI, IGA and Illumina
GrapeReSeq_Illumina_20K_SNP_chip.xls
(http://urgi.versailles.inra.fr/Species/Vitis/GrapeReSeq_Illumina_20K)

Genotyping 2300 samples with the delivered 18K array

INRA-EPGV/CNG platform

2. Chip delivery

A 90% success rate for the bead synthesis

	V. Vinifera set	*Vitis* species set	Chloroplast	Total
No. SNP	13,537	4,510	24	18,071
Average dist. between 2 SNP	31,296	94,059		26,745bp

**Average distance between 2 SNP : chromosome distribution**
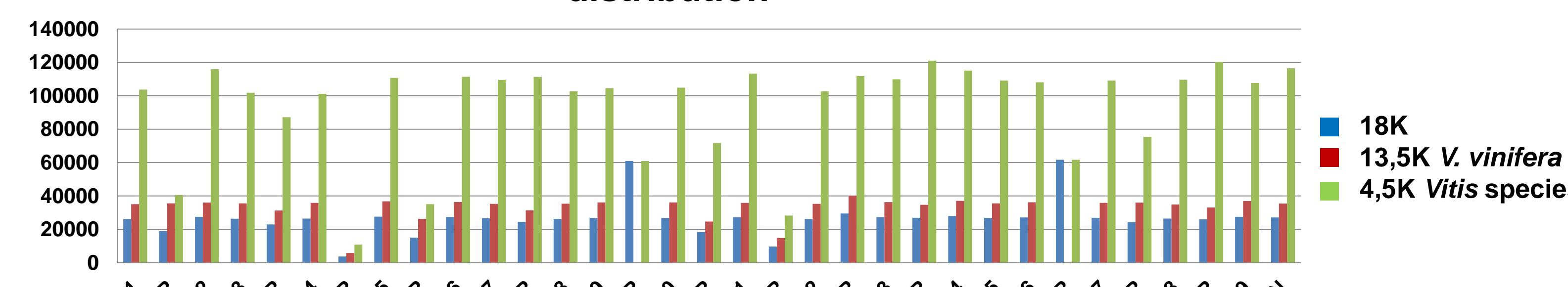
3. Genotyping of 2278 individuals with 18071 SNP

	V. Vinifera progenies	Hybrid progenies	Species diversity	Hybrid diversity	*V. sylvestris* diversity	*v. vinifera* diversity	Total
No. indiv	397	193	135	500	99	954	2278

V. vinifera
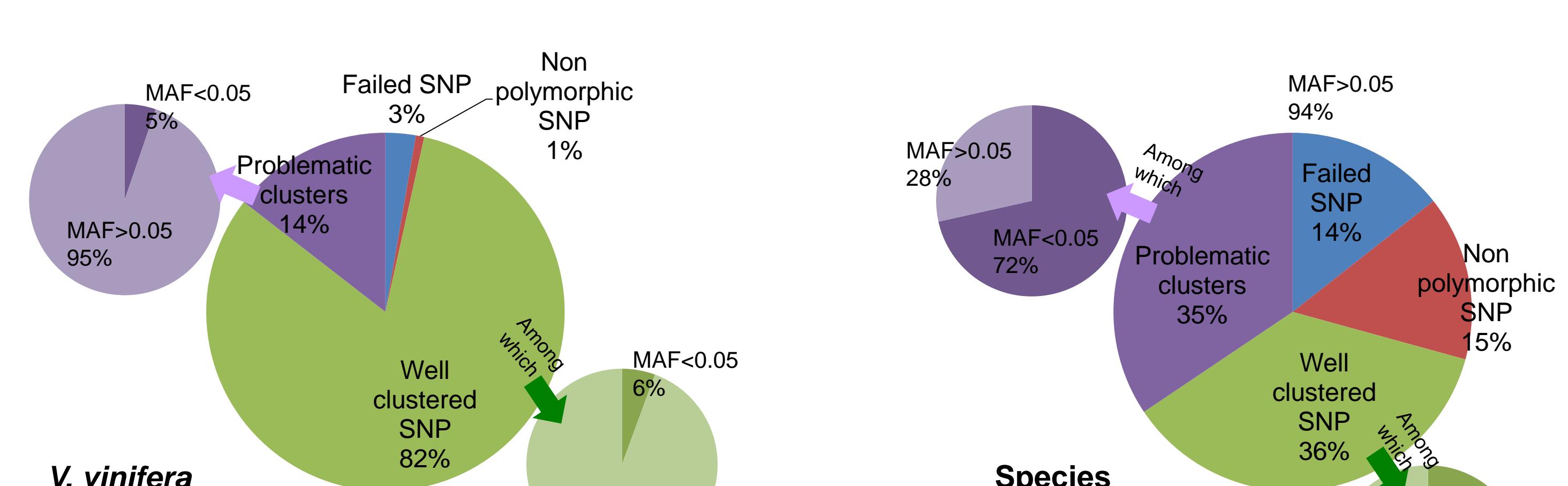
Species

Examples of problematic clusters
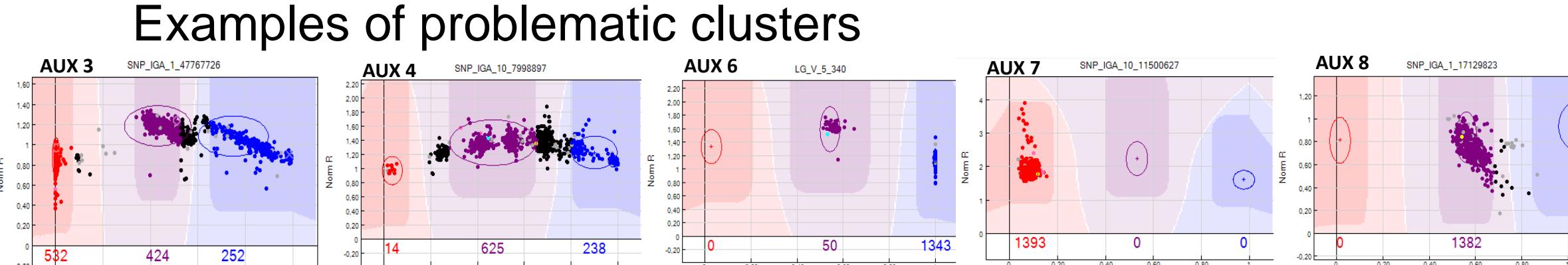
The use of such SNP may depend on the population considered

Cluster file freely available at
http://urgi.versailles.inra.fr/Species/Vitis/GrapeReSeq_Illumina_20K